Title

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Journal

Journal of Industrial Microbiology & Biotechnology, 43(10)

ISSN

1367-5435

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Publication Date

2016-10-01

DOI

10.1007/s10295-016-1809-8

Peer reviewed

Metagenomic applications in environmental monitoring and bioremediation

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space**Abstract** With the rapid advances in sequencing technol- ogy, the cost of sequencing has dramatically dropped and the scale of sequencing projects has increased accordingly. This has provided the opportunity for the routine use of sequencing techniques in the monitoring of environmental microbes. While metagenomic applications have been rou- tinely applied to better understand the ecology and diver- sity of microbes, their use in environmental monitoring and bioremediation is increasingly common. In this review we seek to provide an overview of some of the metagenomic techniques used in environmental systems biology, address- ing their application and limitation. We will also provide several recent examples of the application of metagenomics to bioremediation. We discuss examples where microbial communities have been used to predict the presence and extent of contamination, examples of how metagenomics can be used to characterize the process of natural attenuation by unculturable microbes, as well as examples detailing the use of metagenomics to understand the impact of biostimulation on microbial communities.

Keywords Bioremediation · Metagenomics · Microbial community structure · Environmental systems biology

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space**Introduction**

Bioremediation is a microbially mediated processes employed to degrade and detoxify environmental contaminants [1]. Bioremediation is an appealing approach to dealing with environmental contaminants as it often results in removal of a contaminant through natural biological processes [2]. Much of the research into bioremediation has been focused on understanding the rates of contaminant degradation under natural or perturbed conditions. While microbes are acknowledged as essential to bioremediation, in some cases very little is known about the microbes involved or the impact of various intervention strategies on the microbial community.

Bioremediation approaches can be classified into three main categories separated by the intensity of intervention. Natural attenuation is the least invasive approach, whereby native organisms are used to detoxify contaminants using natural processes. This approach is appealing, as no costly or potentially ecosystem-altering additives are required. However, in many systems the rates of natural attenuation may be prohibitively slow and not responsive to the environmental and health risks. Biostimulation utilizes native organisms, but seeks to increase the rate of biodegradation through relieving some environmental constraints. This is often achieved through the addition of limiting nutrients. In some settings, biostimulation still results in slow rates of biodegradation. These slow biodegradation rates could be due to the inability of the native microbial community to degrade the contaminant of concern. To deal with this issue, non-native organism or enzyme can be added to a system during bioaugmentation in an effort to enhance the rates of biodegradation. This approach is the most invasive, as a non-native organism is added to an ecosystem. However, in some instances bioaugmentation has proven to be

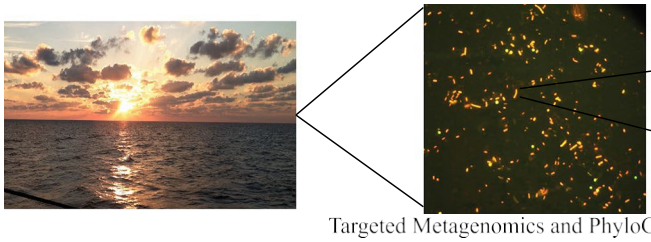
space the most efficient means for remediation [3, 4]. A common concern with bioaugmentation is that non-native organ- isms may not be able to survive under the conditions found in the contaminated system. An additional concern is that these non-native organisms may persist long after the con- taminant has been removed altering the ecosystem.

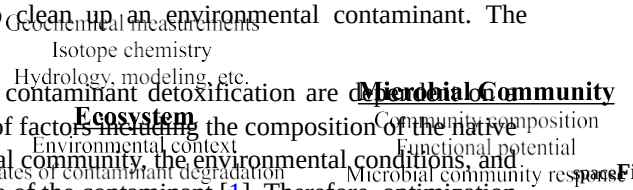
It is important to understand the microbial communities involved in bioremediation and not just the final output and rates of contaminant degradation to most efficiently stimulate the bioremediation processes. Since microbes are the drivers of bioremediation, shifts in the composition and activity of a microbial community may impact the fate of a contaminant in the environment [2]. Recent studies have employed next-generation sequencing approaches to better understand the microbial communities involved in various bioremediation interventions [1]. These approaches have greatly expanded our understanding of the microbial processes involved in bioremediation as well as the impact of various response strategies for contaminant cleanup. The use of molecular biology and metagenomics has also greatly expanded our understanding of the biological sys- tems found in these contaminated environments and in many cases have greatly enhanced our understanding of the

microbial world [2]. Here, we seek to provide a key background on metagenomic approaches and summarize how these tools have been employed to understand contaminated environments in an effort to inform the best practices for environmental cleanup.

Environmental systems biology

The process of bioremediation employs a microbial community to clean up an environmental contaminant. The rates





spaceof contaminant detoxification are dependent on a number of factors including the composition of the native microbial community, the environmental conditions, and the nature of the contaminant [1]. Therefore, optimization of bioreme- diation requires combining complex variables together to understand and predict the fate of environmental contami- nants. Systems biology—the study of the systematic prop- erties and dynamic interactions in a biological system [5, 6]—has been employed to understand complex biological systems and how they w
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in-depth insights into a microbe or micro- bial community. For this reason, environmental systems biology often employs multiple 'omics approaches (e.g., metagenomics, metatranscriptomics, and metaproteomics) to characterize the environmental community in question [6, 9–11] (Fig. 2). Through this approach, various levels

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Fig. 1 Environmental systems biology. Understanding of an environ- mental system involves investigations into each level from ecosys- tems down to individual microbes and molecules. Each level of the

spacesystem can be investigated using different techniques Images pro- vided by Stephen Techtmann and Dominique Joyner

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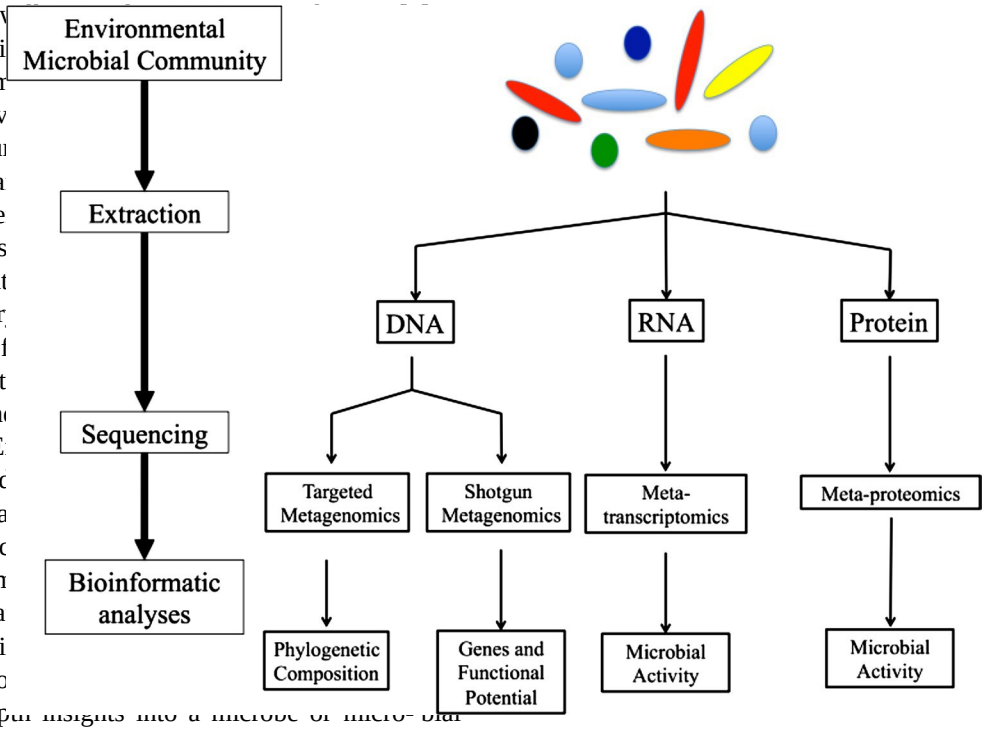

of the system can be interrogated. Sequencing costs have dropped dramatically allowing for more comprehensive characterization of microbial communities and hypothesis-driven experimentation into the response of environmental communities to environmental contaminants. These large sequencing data sets can be incorporated into predictive models to understand how different components of the

system will respond under different conditions. These predictive models have great potential as diagnostic tools to monitor environmental microbial communities and predict responses to various environmental conditions and contaminates [12].

Overview of omics approaches

'Omics approaches are central to environmental systems biology. Metagenomics—the analysis of the total genomic content of a microbial community—has been widely applied to understanding microbial communities in environmental systems (Fig. 2). Other 'omics techniques, including metatranscriptomics (community RNA analysis) and metaproteomics (community protein analysis), have been more recently applied to environmental microbial communities [6]. Here, we attempt to briefly describe some of the 'omics techniques used to study environmental systems focusing on metagenomic approaches (Fig. 2). We also seek to underscore some of the limitations of these techniques to clarify the limits of these approaches.

Metagenomics

Metagenomic approaches often take two forms—targeted metagenomics or shotgun metagenomics (Fig. 2). In targeted metagenomics—or microbiomics—the diversity of a single gene is probed to identify the full complement of sequences of a particular gene in an environment. Targeted metagenomics is most often employed to investigate both the phylogenetic diversity and relative abundance of a particular gene in a sample. This approach is regularly used to investigate the diversity of small subunit rRNA sequences (16S/18S rRNA) in a sample. Microbial ecologists routinely use small subunit rRNA sequencing to understand the taxonomic diversity of an environment. It can also be applied as a tool to investigate the impact of environmental contaminants in altering microbial community structure. To perform targeted metagenomics, environmental DNA is extracted and the gene of interest is PCR amplified using primers designed to amplify the greatest diversity of sequences for that gene of interest. These amplified genes are then sequenced using next-generation sequencing. Next-generation sequencing results in thousands of small subunit rRNA reads per sample and can probe hundreds of samples simultaneously. Targeted metagenomics captures the diversity of single gene of interest, but is limited by the universality of the PCR primers chosen for the analy- sis [13–16]. Furthermore, various bioinformatics analyses have the potential to skew the overall diversity estimates [17]. The strength of targeted metagenomics is that it pro- vides a fairly comprehensive catalog of the microbial taxa present in a set of samples and allows for in-depth com- parison of shifts in microbial diversity before and after a perturbation.

In shotgun metagenomics, the total genomic complement of an environmental community is probed through genomic sequencing (Fig. 2). In this approach, environmental DNA is extracted and then fragmented to prepare sequencing libraries. These libraries are then sequenced to determine the total genomic content of that sample. Shotgun metagenomics is a powerful technique where the functional potential of a microbial community can be identified. Shotgun metagenomics is often most limited by the depth of sequencing. Gaining a complete inventory of the genes in an environmental sample often requires extremely deep sequencing. Good coverage of the entire genomic content of every organism in the community is required for a comprehensive analysis of the functional potential of a community. Oftentimes shotgun metagenomics heavily samples the dominant microbes in a community and only sparsely covers the genomic content of the low abundance members of that community. Furthermore, analysis of metagenomic sequencing data can be very complex as it involves accurately annotating diverse gene sequences, many of which have no homologs in the current sequence databases [18]. The goal of many studies is to link a functional gene with a taxonomic classification using a phylogenetic anchor. This can often be difficult with metagenomics sequencing unless sufficient sequencing depth is achieved and the reads can be accurately assembled into sufficiently long contigs. Many

computational approaches have sought to assemble metagenomic sequences into complete genomes to gain more complete understanding of the functional potential of particular species within a community [19, 20]. Several recent reviews have sought to summarize the key steps in metagenomics and the many potential pitfalls in these techniques [21–24].

In addition to sequencing-based approaches, several microarray-based techniques have been developed [10]. PhyloChip and GeoChip are the two most commonly used microarray technologies. PhyloChip is a 16S rRNA-based microarray able to probe the diversity of 10,993 sub-families in 147 phyla [11]. GeoChip is a functional gene microarray able to probe the diversity of 152,414 genes from

space410 gene categories [25]. Microarray techniques are not dependent on the depth of sequencing to provide compre- hensive insights into the microbial community [10]. They also have the advantage of providing rigorous annotation for the various taxa/genes present on the chip alleviating the limitation of the need for good homologs in the data- base to achieve accurate classification. Microarray-based approaches are, however, limited in that only the genes on the chip can be detected, thus limiting the potential for discovery of new genes or pathways in a sample. Micro- array-based approaches are often a helpful complement to sequencing-based approaches as an additional line of evidence.

Metatranscriptomics and metaproteomics

Metatranscriptomics and metaproteomics are increasingly being applied to environmental systems (Fig. 2). These approaches provide key insights into the actively expressed genes in a microbial community and are thus good indica- tors for the microbial functions being expressed under the conditions at the time of sampling. In metatranscriptomics, RNA is extracted from an environmental sample. The RNA is converted into cDNA and sequenced in a similar fashion to metagenomics (Fig. 2). This approach provides an inven- tory of the actively expressed genes in a sample. Metaprot- eomics does not involve nucleic acid sequencing, but rather high-resolution mass spectrometry combined with enzy- matic digests of proteins and liquid chromatography [26]. Metaproteomics provides insights into the complement of proteins found in an environmental sample including post-translational modifications in proteins that may impact their activity. Several reviews have summarized the strengths and weakness of these techniques [23, 26, 27].

Case studies

To clarify how metagenomics can be applied to bioremediation applications and environmental monitoring, we will discuss three case studies that exemplify the application of these techniques. These recent studies have employed both targeted and shotgun metagenomics as tools for environ- mental monitoring as well as to assess the impacts of vari- ous remediation interventions—namely, natural attenuation and biostimulation.

Case study 1: microbial communities as environmental sensors

A central tenant of ecological theory is that ecological forces practicably restrict or promote the growth of particular taxa according to the environmental conditions

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[28]. These conditions may be due to natural fluctuations or anthropogenic activity such as contamination. Based on this finding, many studies have sought to use commu- nity members as indicator species or biosensors of par- ticular environmental features. A recent study sought to use machine learning as the basis for developing a model capable of predicting the environmental conditions based on the microbial community structure [12]. This model was specifically designed to investigate the ability of microbial communities to predict the presence of particular contaminants in uranium and nitrate-contaminated groundwater as well as oil-contaminated marine samples. This study used two data sets as the basis for their predictive model. Both data sets assessed the taxonomic diversity of the microbial population using 16S rRNA genes. One data set was from groundwater samples collected from a uranium and nitrate-contaminated aquifer from the Bear Creek watershed in Oak Ridge, Tennessee [29]. Many field studies have been undertaken in this location to assess the potential for uranium reduction as a means of immobilization and remediation [30–37]. Several studies have investigated the potential for bioremediation as a means to stimulate uranium reduction. Due to the need for monitoring of the contaminated groundwater plume, a number of wells have been dug to monitor the progression of the contaminants in the groundwater. Across these wells, there is a dramatic range of environmental conditions [38]. Uranium and nitrate are two of the primary contaminants in this aquifer. Ninety-three wells were sampled for this study and the geochemistry and microbial community structure were determined for each well [9]. In these wells, the uranium concentrations ranged from non-detectable to 55.3 mg/L. The nitrate concentrations ranged from non-detectable to 14,446 mg/L.

Microbial community structure was probed using high-throughput 16S rRNA sequencing. These sequencing data were analyzed to determine the taxonomic composition of these communities and the relative abundance of each taxon. A computation model was built which sought to relate the microbial community structure to the geochemical variables. This computational model was trained against a subset of the data and then validated against the remaining data. This validation process involved submitting a microbial community profile to the algorithm and testing whether the model's prediction matched the geochemical

measurement. The model was relatively good at predicting the level of uranium and nitrate in these samples. Furthermore, there were key taxa that were shown to be highly indicative of particular ranges of geochemical variables. Many of the key taxa that were indicative of a particular contaminant were related to taxa involved in the metabolism of the contaminant. For example, *Brevundimonas* spp. were shown to be some of the most informative features for identifying nitrate-contaminated wells. *Brevundimonas* spp.

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have been shown to be active nitrate reducers in groundwa- ter [39]. Additionally, *Rhodanobacter* and *Rhodocyclaceae* were key features for predicting uranium. Both of these taxa have been previously identified as being involved in uranium reduction and bioremediation [40].

To further validate the utility of this model, the model's ability to predict the presence of oil contamination in the marine environment was determined. In this portion of the study, 16S rRNA microarray (PhyloChip) data were used. Samples were collected from the Gulf of Mexico during the *Deepwater Horizon* oil spill [41]. These samples were analyzed using the PhyloChip to determine the taxonomic composition of each sample and the relative abundance of each taxon. Along with the microbial community samples, oil concentrations were measured. These samples could be grouped into three categories—oil contaminated, non-con- taminated, or post-contamination. Post-contaminated sam- ples were collected from sites that at one point during the spill had measurable levels of oil, but at the time of sam- pling had no detectable oil based on GC/MS. The micro- bial community profiles and oil data were used to build a model to distinguish between these three conditions. This model was nearly perfect in its ability to bin samples into these three categories. Furthermore, it was shown that two microbial groups were sufficient to distinguish between these three categories. *Oceanospirillales* were good indi- cators of the presence of oil contamination, while *Pelagi- bacteriaceae* were good indicators of non-contaminated sites. This study provides further support for the ability of metagenomic data to be employed as a means for monitor- ing the presence and extent of contamination in groundwa- ter as well as in the marine environment.

Case study 2: marine oil biodegradation (*Deepwater Horizon* oil spill)

Oil is a natural part of the marine system [42]. However due to increased anthropogenic activity, accidental oil spills have impacted a number of marine settings [43, 44]. The *Deepwater Horizon* oil spill is the worst marine oil spill in the USA [45]. Approximately, 4.1 million barrels of oil were released into the Gulf of Mexico [46, 47]. Many microbes possess the ability to degrade the components of crude oil [43, 48, 49]. As such, oil spill bioremediation has been applied in a number of systems. The *Deepwater Hori- zon* oil spill was one of the first marine oil spills in which metagenomics were extensively applied to better under- stand the fate of oil and the mechanism of oil biodegrada- tion in the marine environment.

A number of studies sought to understand the response of the microbial community in the Gulf of Mexico to the released oil. The *Deepwater Horizon* oil spill was unique in that a portion of the oil remained trapped in the deep

space ocean and was known as a deep water plume of oil [11, 50]. The conditions within this deep water plume were quite distinct from the conditions found in the surface slick. Many groups used targeted metagenomics as a tool to investigate the differences in the microbial community response to oil in these two distinct settings [51, 52]. It was shown that the microbial community in the surface water was composed primarily of *Cycloclasticus, Alteromonas, Halomonas,* and *Pseudoalteromonas* [51, 52]. However, the microbial community in the deep water was primarily composed of psychrophilic oil-degrading microbes related to *Oceanospirillales*, *Colwellia*, and *Cycloclasticus* [11, 51]. In some samples during the early time points of the spill, a single operational taxonomic unit (OTU) related to *Oceanospirillales* comprised greater than 90 % of the microbial community in oil-impacted deep water [53]. This *Oceanospirillales* sp. resisted isolation. Therefore the use of metagenomics was the key method for under- standing its role in deep water oil biodegradation. Shotgun metagenomic sequencing was applied to samples collected during the *Deepwater Horizon* oil spill [53]. This analy- sis revealed that a diverse set of genes including genes for chemotaxis and hydrocarbon degradation were enriched in samples from the deep water plume compared to uncon- taminated deep water. Additionally, the genes for degrada- tion of BTEX compounds were expressed at relatively low levels in the plume. The use of single cell genomics shed light on the role of the dominant *Oceanospirillales* sp. in the deep water plume. Evidence from the single ampli- fied genomes revealed the presence of genes involved in *n*-alkane and cycloalkane degradation. This indicates that at early time points in the spill, the dominant physiologies were those involved in degradation of alkanes. There were distinct shifts in the microbial community during the spill as assessed by PhyloChip data [41]. This shift resulted in a microbial community more adept at the degradation of the more recalcitrant aromatic compounds. These dynam- ics in community structure corresponded to the extent and quality of oil input into the system. The use of metagen- omic sequencing during the *Deepwater Horizon* oil spill resulted in dramatic advances in our understanding of the microbial community response to released oil in the marine environment. Furthermore, the uses of metagenomics led to the identification of a great diversity of cold-adapted oil-degrading bacteria and clarified their role in remediating oil pollution in cold environments.

Case study 3: biostimulation to increase uranium oxidation in a contaminate aquifer

A large portion of the population relies on groundwater for drinking water. However, groundwater is susceptible to contamination. Bioremediation has been proposed as spaceone means of dealing with groundwater contamination. In addition to organic contaminates, heavy metals can also contaminate groundwater environments. Uranium-con- taminated groundwater is of great concern due to releases associated with mining, milling, processing as well as from natural sources [54]. Microbes have the ability to interact with uranium and help to limit the impacts of uranium con- tamination. The mobility of uranium depends on its specia- tion and redox state [55]. U(VI) is soluble in aqueous solu- tions, whereas U(IV) is insoluble. Many metal-reducing bacteria are able to reduce U(VI) to U(IV) using hydrogen, lactate, acetate, and ethanol among many others as electron donors [56]. Therefore, metal-reducing microbes have been proposed as a means of remediating uranium-contaminated sites by limiting the spread of soluble uranium. Uranium reduction in groundwater can be stimulated by the addition of an electron donor [35, 36, 57].

A number of studies in two different uranium-contaminated aquifers have employed metagenomics to test the impact of biostimulation on uranium reduction and the key microbes involved in uranium reduction in groundwater. These studies tested stimulation with acetate and ethanol as well as stimulation with emulsified vegetable oil. A num- ber of studies were performed at the US DOE Rifle site in Colorado. At the Rifle site, mill tailings from uranium processing mine leached into the groundwater [58]. The microbial community at the Rifle site has been extensively studied using metagenomic techniques. The microbial community in background groundwater was highly diverse. Biostimulation of uranium reduction through amendment with acetate was tested in 2002 [58]. During bioreduction of uranium, the microbial community was dominated by *Geobacter* spp. [58–60]. To understand which microbes in the community were consuming the amended acetate, stable isotope probing combined with analysis of the 16S rRNA gene was employed with ^{13}C-labeled acetate. This indicated that *Geobacter* spp. were the primary organisms utilizing the acetate and incorporating the acetate into the biomass [61]. Whole genome microarray analysis of *Geobacter uraniireducens* indicated that expression of *rpsC* is a good indicator of growth rates. This was then confirmed in situ during acetate stimulation at the Rifle site, which demonstrated that analysis of expression of *rpsC* is indicative of the actual rate of *Geobacter* species growth and metabolism [62]. Proteomic analysis of the planktonic microbial community in the acetate-stimulated groundwater indicated a dominance of *Geobacter* proteins related to acetate metabolism and energy generation [63].

Using 16S rRNA analysis, it was demonstrated that biostimulation resulted in a decrease in the overall diversity of species in the groundwater. There was, however, an increase in microbial taxa believed to be involved in iron and sulfur cycling based on PhyloChip analysis [64].

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During stimulation, a distinct shift was observed from dominance by iron reducers to a community dominated by sulfate reducers [65]. Changes in the functional diversity of the groundwater microbial community during acetate biostimulation were monitored using GeoChip [66]. GeoChip analysis demonstrated that during acetate amendment, the microbial community proceeded from being dominated by pathways involved in iron reduction to sulfate reduction pathways, and finally to methanogenic pathways. These data confirm that during amendment, there is a distinct progression of communities with different physiologies. This progression was also observed through metaproteomic analysis [67].

Similar amendments were performed at the Oak Ridge Field Research Center (ORFRC) site in Tennessee. This site was contaminated with uranium during disposal of waste into unlined ponds and is part of the locations sam- pled in case study 1. The groundwater at the ORFRC is of low pH and contaminated with both uranium and nitrate [29]. Similar to the Rifle site, the microbial communities in contaminated locations had lower diversity than in the background sites [68]. The microbial community structure was determined through 16S rRNA analysis of wells during stimulation with the addition of ethanol [69]. This work demonstrated that known uranium reducers were present in the stimulated groundwater up to 2 years after amend- ment. Indicator species analysis combined with massively parallel 16S rRNA sequencing identified a strong associa- tion between certain taxa of sulfate-, Fe(III)-, and U(VI)-reducing bacteria and sites of active U(VI) reduction during ethanol amendment [70].

The addition of electron donors, such as ethanol and acetate, was shown to stimulate the bioreduction of uranium. However, to achieve long-term reduction of uranium, several injections of electron donor were required. An in-depth analysis of the impact of emulsified vegetable oil (EVO) on stimulating uranium bioreduction was undertaken. EVO is an appealing amendment for its high energy content and relatively slow degradation rate allowing for sustained reduction of uranium after a single amendment [71]. After amendment with EVO, uranium levels remained below the pre-injection levels for 269 days [37]. During this time, the microbial community consumed EVO. 16S rRNA sequencing was performed to assess the impact of this amendment on the microbial community. EVO amendment resulted in long-term alteration in the microbial community in the aquifer. The diversity of the microbial community in the groundwater was dramatically reduced after amendment with EVO in a similar fashion to amendment with acetate at the Rifle site. Despite the decrease in diversity, there was a substantial increase in the microbial abundance. During the

monitoring period after injection, the environmental conditions returned to pre-injection levels. However, the space
microbial community did not return to the composition found in the wells prior to injection. The EVO-amended community had high levels of *Geobacter* spp. similar to the acetate-stimulated community at the Rifle site. However, the EVO-stimulated community also had high levels of *Pelosinus* and *Desulforegula* spp. *Pelosinus* species are believed to be involved in the fermentation of the EVO. The fermentation products are then able to stimulate other community members. Furthermore, *Pelosinus* strain UFO1, which was isolated from non-contaminated sediments at the Oak Ridge site, is able to reduce U(VI) [72, 73]. There- fore, *Pelosinus* spp. may be able to ferment EVO as well as aid in uranium reduction.

GeoChip analysis was also performed during and after the EVO amendment to better understand the impact of stimulation by EVO. Sequential shifts in the functional potential of the microbial community were observed [74]. Some of these shifts involved changes in the expression of genes involved in EVO degradation. Additionally, genes
involved in reduction of NO_3^-, Mn(IV), Fe(III), U(VI), and SO_4^- were enriched post-injection, especially during times
of peak U(VI) reduction.

The use of 16S rRNA sequencing and metagenomics has greatly expanded our understanding of the ability of microbes to reduce uranium during stimulation with electron donor. Amendment with electron donor often results in decreased diversity and enrichment of microbe able to reduce metals. While *Geobacter* spp. appears to be important uranium reducers in many environments, *Pelosinus* spp. are also an important uranium reducer especially during amendment with EVO. Furthermore, the use of metagenomics clarifies the succession of important metabolic physiologies during and after electron donor addition.

Conclusions and next steps

As sequencing costs continue to decrease, the utility of high-throughput 16S rRNA sequencing and metagenomics increases. These approaches allow for in-depth examination into the effect of various bioremediation interventions on the native microbial community. This allows for optimization of these techniques to enriched targeted groups of microbes most important to the bioremediation process. Furthermore, these approaches allow for a more unbiased analysis of the microbial community, capturing microbes that resist culturing. Work during the *Deepwater Horizon* oil spill identified the importance of certain groups of uncultured microbes in the biodegradation of oil. Metagen- omics has the potential to inform the appropriate use of remediation strategies to accomplish rapid remediation of a contaminant in a minimally invasive manner. Metagenomic approaches also enable a mechanistic understanding of the

space processes involved in bioremediation, which will inform efforts to optimize the efficacy of bioremediation.

Moving forward, there is a great need to fully understand the key taxa and pathways involved in many of these processes. The ease of sequencing has resulted in massive amounts of data leading to the discovery of many uncultured phyla and gene families with no known function [17]. This holds true for contaminated environments as well. There is a great need to combine metagenomic approaches with classical culture-based approaches to more fully understand the microbes involved in these processes. Current approaches often study the changes in microbial diversity or gene diversity in response to a perturbation. This provides lists of genes and taxa that respond to amendments. However, mechanistic understanding of the community response or the important biochemical pathways involved in responding to these perturbations is dependent on genetic and biochemical analyses performed on model organisms. Often, these model organisms are distantly related to the key taxa in these environments. Efforts to isolate environmentally relevant taxa from the environments of interest will greatly expand our understanding of the metagenomic data sets gen- erated from these sites. The use of 16S rRNA sequencing and metagenomics has great potential to inform bioremedia- tion strategies and provide deep insights into the microbial response to contamination or remediation techniques. As these approaches are combined with pure-culture analysis of environmentally relevant microbes, a more complete pic- ture of the basis for bioremediation will be obtained.

Acknowledgments This work was in part supported by contract A13-0119-001 Deep Sea Basin Microbiology between the University of Tennessee and BP.

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